

VI. CLAIMS

We claim:

1. A method of generating a sperm cell insemination sample, comprising:
 - 5 a. obtaining semen from a male of a species of mammal;
 - b. generating a fluid stream having flow characteristics;
 - c. altering flow characteristics of said fluid stream to adjust fluid stream pressure;
 - d. entraining said sperm cells into said fluid stream;
 - 10 e. controlling sperm cell fertility characteristics through adjustment of said fluid stream pressure; and
 - f. generating a sperm cell insemination sample having controlled sperm cell fertility characteristics.
- 15 2. A method of generating a sperm cell insemination sample a described in claim 1, where in said species of mammal is selected from the group consisting of a bovine species of mammal, an equine species of mammal, an ovine species of mammal, a canine species of mammal, a feline species of mammal, a swine species of mammal, a marine species of mammal, a deer species of mammal, a primate species of mammal, a goat species of mammal.
- 20 3. A method of generating a sperm cell insemination sample a described in claim 1, wherein said fluid stream comprises a sheath fluid stream.
- 25 4. A method of generating a sperm cell insemination sample a described in claim 3, wherein said sheath fluid stream comprises a sheath fluid containing phosphate buffered saline (PBS).
- 30 5. A method of generating a sperm cell insemination sample a described in claim 3, wherein said sheath fluid stream comprises a sheath fluid containing a citrate buffer.
6. A method of generating a sperm cell insemination sample a described in claim 5, wherein said citrate buffer comprises about 2.9% sodium citrate.

7. A method of generating a sperm cell insemination sample a described in claim 3, wherein said sheath fluid stream comprises a sheath fluid containing a HEPES buffer.

8. A method of generating a sperm cell insemination sample a described in claim 1, 5 wherein said fluid stream is generated within a flow cytometer or cell sorter.

9. A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of altering flow characteristics of said fluid stream to adjust fluid stream pressure comprises altering said flow characteristics to adjust said fluid stream pressure to 10 between about 20 pounds per square inch and about 60 pounds per square inch.

10. A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining bovine semen from said male of a bovine species of mammal and said step of 15 controlling sperm cell fertility characteristics through adjustment of said fluid stream pressure comprises establishing bovine sperm cell fertility characteristics of bovine sperm cells entrained within said fluid stream which are not substantially different than said bovine sperm cell fertility characteristics of said bovine sperm cells in said semen.

20 11. A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining bovine semen from said male of a bovine species of mammal and said step of controlling sperm cell fertility characteristics through adjustment of said fluid stream pressure comprises establishing said bovine sperm cell fertility characteristics of said 25 bovine sperm cells entrained within said fluid stream which are substantially comparable said bovine sperm cell fertility characteristics of said bovine sperm cells in said semen.

12. A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises 30 obtaining said bovine semen from said male of a bovine species of mammal and said step of generating a sperm cell insemination sample having controlled sperm cell fertility characteristics comprises generating a bovine sperm cell insemination sample having said bovine sperm cell fertility characteristics which are not substantially different than bovine sperm cell fertility characteristics of said bovine sperm cells in said bovine semen.

13. A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said bovine semen from said male of a bovine species of mammal and said step 5 of generating a sperm cell insemination sample having controlled sperm cell fertility characteristics comprises generating a bovine sperm cell insemination sample having bovine sperm cell fertility characteristics which are substantially comparable to bovine sperm cell fertility characteristics of said bovine sperm cells in said bovine semen.

10 14. A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said bovine semen from said male of a bovine species of mammal and said step of altering flow characteristics of said fluid stream to adjust fluid stream pressure comprises adjusting said flow characteristics of said fluid stream to adjust fluid stream 15 pressure to between about 30 pounds per square inch and about 50 pounds per square inch.

15. A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said bovine semen from said male of a bovine species of mammal and said step 20 of altering flow characteristics of said fluid stream to adjust fluid stream pressure comprises adjusting said flow characteristics of said fluid stream to adjust fluid stream pressure to between about 30 pounds per square inch and about 40 pounds per square inch.

16. A method of generating a sperm cell insemination sample a described in claim 1, 25 wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said bovine semen from said male of a bovine species of mammal and said step of altering flow characteristics of said fluid stream to adjust fluid stream pressure comprises adjusting said flow characteristics of said fluid stream to adjust fluid stream pressure to about 40 pounds per square inch.

30 17. A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining equine semen from said male of a equine species of mammal and said step of controlling sperm cell fertility characteristics through adjustment of said fluid stream

pressure comprises establishing equine sperm cell fertility characteristics of equine sperm cells entrained within said fluid stream which are not substantially different than said equine sperm cell fertility characteristics of said equine sperm cells in said semen.

5 18. A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining equine semen from said male of a equine species of mammal and said step of controlling sperm cell fertility characteristics through adjustment of said fluid stream pressure comprises establishing said equine sperm cell fertility characteristics of said equine sperm cells entrained within said fluid stream which are substantially comparable said equine sperm cell fertility characteristics of said equine sperm cells in said semen.

10 19. A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said equine semen from said male of a equine species of mammal and said step of generating a sperm cell insemination sample having controlled sperm cell fertility characteristics comprises generating a equine sperm cell insemination sample having said equine sperm cell fertility characteristics which are not substantially different than equine sperm cell fertility characteristics of said equine sperm cells in said equine semen.

20 20. A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said equine semen from said male of a equine species of mammal and said step of generating a sperm cell insemination sample having controlled sperm cell fertility characteristics comprises generating a equine sperm cell insemination sample having equine sperm cell fertility characteristics which are substantially comparable to equine sperm cell fertility characteristics of said equine sperm cells in said equine semen.

25 21. A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said equine semen from said male of a equine species of mammal and said step of altering flow characteristics of said fluid stream to adjust fluid stream pressure comprises adjusting said flow characteristics of said fluid stream to adjust fluid stream pressure to between about 30 pounds per square inch and about 50 pounds per square inch.

22. A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said equine semen from said male of a equine species of mammal and said step 5 of altering flow characteristics of said fluid stream to adjust fluid stream pressure comprises adjusting said flow characteristics of said fluid stream to adjust fluid stream pressure to between about 30 pounds per square inch and about 40 pounds per square inch.

23. A method of generating a sperm cell insemination sample a described in claim 1, 10 wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said equine semen from said male of a equine species of mammal and said step of altering flow characteristics of said fluid stream to adjust fluid stream pressure comprises adjusting said flow characteristics of said fluid stream to adjust fluid stream pressure to about 40 pounds per square inch.

15 24. A method of generating a sperm cell insemination sample a described in claim 1, wherein one of said sperm cell fertility characteristics comprises sperm cell motility.

25. A method of generating a sperm cell insemination sample a described in claim 1, 20 wherein said fertility characteristics comprises bovine sperm cell motility.

26. A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises equine sperm cell motility.

25 27. A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises sperm cell viability.

28. A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises bovine sperm cell viability.

30 29. A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises equine sperm cell viability.

30. A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises pregnancy rate of a female of said species of mammal inseminated with said sperm cell insemination sample having controlled sperm cell fertility characteristics.

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31. A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises pregnancy rate of said female of said bovine species of mammal inseminated with a bovine sperm cell insemination sample having controlled sperm cell fertility characteristics.

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32. A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises pregnancy rate of said female of said equine species of mammal inseminated with an equine sperm cell insemination sample having controlled sperm cell fertility characteristics.

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33. A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises cleavage rate of oocytes fertilized with said sperm cell insemination sample having controlled sperm cell fertility characteristics.

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34. A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises cleavage rate of bovine oocytes fertilized with said bovine sperm cell insemination sample having controlled sperm cell fertility characteristics.

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35. A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises cleavage rate of equine oocytes fertilized with said equine sperm cell insemination sample having controlled sperm cell fertility characteristics.

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36. A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises blastocyst rate of oocytes fertilized with said sperm cell insemination sample having controlled sperm cell fertility characteristics.

37. A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises blastocyst rate of bovine oocytes fertilized with said bovine sperm cell insemination sample having controlled sperm cell fertility characteristics.

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38. A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises blastocyst rate of equine oocytes fertilized with said equine sperm cell insemination sample having controlled sperm cell fertility characteristics.

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39. A method of generating a sperm cell insemination sample a described in claim 31, wherein said bovine sperm cell insemination sample having controlled sperm cell fertility characteristics contains between about 1×10^5 and 2×10^7 of said bovine sperm cells.

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40. A method of generating a sperm cell insemination sample a described in claim 31, wherein said bovine sperm cell insemination sample having controlled sperm cell fertility characteristics contains between about 1×10^6 and 3×10^6 of said bovine sperm cells.

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41. A method of generating a sperm cell insemination sample a described in claim 1, further comprising the step of staining said sperm cells.

42. A method of generating a sperm cell insemination sample a described in claim 41, wherein said step of staining said sperm cells comprises staining said sperm cells with Hoechst 33342.

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43. A method of generating a sperm cell insemination sample a described in claim 1, further comprising generating droplets in said fluid stream some of which contain one each of said sperm cells.

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44. A method of generating a sperm cell insemination sample a described in claim 43, further comprising differentiating said sperm cells based upon a sex characteristic.

45. A method of generating a sperm cell insemination sample a described in claim 44, wherein said step of differentiating said sperm cells based on a sex characteristic comprises differentiating said sperm cells based on amount of DNA content.

5 46. A method of generating a sperm cell insemination sample a described in claim 44, wherein said step of differentiating said sperm cells based on a sex characteristic comprises differentiating said sperm cells based on sperm head volume.

10 47. A method of generating a sperm cell insemination sample a described in claim 44, further comprising the step of separating said sperm cells based on said sex characteristic.

48. A method of generating a sperm cell insemination sample a described in claim 47, further comprising the step of collecting said sperm cell insemination sample having controlled sperm cell fertility characteristics in a collection container.

15 49. A method of generating a sperm cell insemination sample a described in claim 48, wherein said step of collecting said sperm cell insemination sample having controlled sperm cell fertility characteristics in a collection container comprises collecting a sex selected sperm cell insemination sample in said collection container.

20 50. A method of generating a sperm cell insemination sample a described in claim 49, wherein said sex selected sperm cell insemination sample comprises an artificial insemination sex selected sperm cell insemination sample.

25 51. A method of generating a sperm cell insemination sample a described in claim 49, wherein said sex selected sperm cell insemination sample comprises an in vitro fertilization sex selected sperm cell insemination sample.

52. A method of generating a sperm cell insemination sample a described in claim 49, 30 wherein said sex selected sperm cell insemination sample comprises an intracytoplasmic sex selected sperm cell injection sample.

53. A method of assessing sex selected sperm cell fertility, comprising:
a. obtaining sperm cells from a male of a species of mammal;

- b. providing Y-chromosome bearing sex selected sperm cells exposed to first treatment conditions;
- c. providing X-chromosome bearing sex selected sperm cells exposed to second treatment conditions;
- 5 d. generating an insemination sample having a ratio of said Y-chromosome bearing sex selected sperm cells exposed to said first treatment conditions and said X-chromosome bearing sex selected sperm cells exposed to second treatment conditions;
- e. inseminating at least one female of said species of mammal with said insemination sample having said ratio of said Y-chromosome bearing sperm cells exposed to said first treatment conditions and said X-chromosome bearing sperm cells exposed to second treatment conditions;
- 10 f. producing offspring from said at least one female of said species of mammal;
- g. assessing fertility of said sex selected sperm cells by comparison of sex ratio of offspring of said at least one female of said species of mammal to said ratio of said Y-chromosome bearing sperm cells exposed to first treatment conditions and X-chromosome bearing sperm cells exposed to second treatment conditions

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54. A method of assessing sex selected sperm cell fertility as described in claim 53, wherein said ratio of said Y-chromosome bearing sperm cells exposed to said first treatment conditions and said X-chromosome bearing sperm cells exposed to second treatment conditions comprises substantially equal numbers of Y-chromosome bearing sperm cells exposed to said first treatment conditions and said X-chromosome bearing sperm cells exposed to second treatment conditions.

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30 55. A method of assessing sex selected sperm cell fertility as described in claim 53, wherein said sex ratio of offspring of said at least one female of said species of mammal comprises a sex ratio of embryos.

56. A method of assessing sex selected sperm cell fertility as described in claim 53, wherein said sex ratio of offspring of said at least one female of said species of mammal comprises a sex ratio of fetuses.

56. A method of assessing sex selected sperm cell fertility as described in claim 53, wherein said sex ratio of offspring of said at least one female of said species of mammal comprises a sex ratio of offspring born.

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57. A method of assessing sex selected sperm cell fertility as described in claim 53, wherein said at least one female of said species of said mammal comprises a sufficient number of females to of said species to produce sufficient numbers of offspring to establish said sex ratio of offspring.

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58. A method of assessing fertility of sperm cells from a male of a species of mammal, comprising:

- a. obtaining sperm cells from a least two males of a species of mammal;
- b. exposing said sperm cells from each of said at least two males of said species to substantially the same flow cytometric treatment;
- c. inseminating at least one female of said species of mammal with a mixture of substantially equal numbers of said sperm cells from each of said at least two males of said species of mammal exposed to substantially the same flow cytometric treatment;
- 15 d. collecting embryos from said at least one female of said species of mammal;
- e. determining which of said at least two males of said species of mammal sired each embryo; and
- f. ranking comparative fertility of said at least two males of said species of mammal based on comparative number of embryos sired by each of said at least two males.

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59. A method of assessing fertility of sperm cells from a male of a species of mammal as described in claim 58, wherein said flow cytometric treatment comprises separating said sperm cells from each of said at least two males based upon a sex characteristic.

60. A method of assessing fertility of sperm cells from a male of a species of mammal as described in claim 58, wherein said flow cytometric treatment comprises sex selecting sperm cells from each of said at least two males.

61. A method of assessing fertility of sperm cells from a male of a species of mammal, comprising:

- 5 a. obtaining sperm cells from a least two males of a species of mammal;
- b. exposing said sperm cells from each of said at least two males of said species to substantially the same flow cytometric treatment;
- c. fertilizing oocytes in vitro with a mixture of substantially equal numbers of said sperm cells from each of said at least two males of said species of mammal exposed to substantially the same flow cytometric treatment;
- 10 d. collecting embryos generated through said in vitro fertilization of said oocytes;
- e. determining which of said at least two males of said species of mammal sired each embryo; and
- f. ranking comparative fertility of said at least two males of said species of mammal based on comparative number of embryos sired by each of said at 15 least two males.